MATERNAL CARE, MALE-MALE AGGRESSION, AND THE USE OF A SPECIALIZED APPENDAGE IN THE CAPRELLID AMPHIPOD, *CAPRELLA MUTICA*

by

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Aggressive behaviors in many animals increase juvenile survival and reproductive success. I explored the role of aggressive behavior in maternal care and male-male competition in caprellid amphipods. Attacks of females in three reproductive states toward male and female conspecific and congeneric caprellids were quantified. Parental females showed greatest aggression toward males of both species, suggesting these caprellids may pose threats to juveniles. Paired males were exposed to females in three reproductive states and other males and fought most often in the presence of non-brooding females. This finding suggests that males recognize and fight over receptive females. Males fight with the second gnathopods and "poison tooth," which may cause fatalities in conflicts. Mortality rates of males with and without (removed) poison teeth were not significantly different, but the highest observed mortality rate was no-tooth

caprellids when paired with toothed males, suggesting presence of the teeth may impact the outcome of male-male conflicts.

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CHAPTER I

GENERAL INTRODUCTION

Aggressive behaviors in many adult animals have been shown to increase their reproductive success and the survival of their offspring. Of the aggressive behaviors, I have investigated maternal care and male-male aggression. Maternal care can provide juveniles with protection from predation, and male-male aggression can increase the dominant male's mating opportunities. In some cases, weaponry can assist animals in eliminating threats or competition. The "poison tooth," a protrusion from the palmar surface of the second gnathopods, is thought to be used as a weapon in male caprellid amphipods. In this study, I investigate the function of maternal care and male-male aggression in caprellid amphipods, along with the role the poison tooth plays in mortalities in aggressive encounters between males.

Many species of animals provide care for their offspring. Parental care can enhance survivorship of offspring at varying stages of development, ranging from the production of large yolky eggs through the protection of juveniles from predators (Clutton-Brock 1991). This care can be characterized by alleviation of physiological stress, supplying food, and protection from predators; consequently, we would expect parental care to be highest in environments where there is high competition for resources or considerable predation (Clutton-Brock 1991). It is hypothesized that the female, rather

than the male, usually provides care because it is more energetically costly to produce oocytes than sperm (Trivers 1972). In this case, she would be providing care to protect her past investments. However, this higher cost in females should not be viewed as a past investment, but as contributing to the high cost of producing more offspring, as opposed to providing care for the present offspring (Maynard Smith 1977). Maternal care has been most commonly studied in mammals (Maestripieri 1992; Ebensperger 1998), birds (Regelmann and Curio 1983; Montgomerie and Weatherhead 1988; Pierotti 1991), and insects (Tallamy and Brown 1999).

Infanticide from conspecifics is very common in mammals. The young of many mammals are vulnerable and rely on the parental female for protection (Ebensperger 1998). Southern elephant seal females were more aggressive and successful in fights with conspecifics after giving birth than before (McCann 1982). This aggression may be provided to protect pups. Aggression in female mice increases postpartum, and it has been experimentally shown that maternal care greatly increases the survival of the juveniles (Wolff 1985).

Maternal care also increases offspring survival in amphibians and reptiles (Bickford 2004; Huang 2006). Some frog species that benefit from maternal care provide protection from physiological stresses or predation for embryos depending on their habitat (Bickford 2004). *Hylophorbus rufescens*, a terrestrial frog, guards the eggs all day against the threat of ant predation. *Oreophyrne* sp. "A", an arboreal frog, remains with its clutch only part time. Their eggs are threatened by desiccation, so parental frogs come back to hydrate the eggs everyday, but continuous care is not required (Bickford 2004). Mortality of eggs in unattended clutches from *H. rufescens* and *Oreophyrne* sp. "A" was

100%, whereas mortality rates of eggs attended by mothers were 22% and 0%, respectively (Bickford 2004). Huang (2006) showed that there is a great benefit for the skink *Mabuya longicaudata* in protecting its eggs from snake predators; median hatching success for guarded nests was 81%, while median hatching success for unguarded nests was 18%. This skink can identify and fight species that pose a threat to their offspring, escape from their own predators, and ignore all other species (including conspecifics) that do not pose a threat to the mother or her offspring (Huang 2006). These examples illustrate great benefits in maternal care and recognition systems.

Insects, due to their diverse life histories, can aid the development of their offspring in various ways. Maternal thornbug treehoppers, *Umbonia crassicornis*, protect their young from wasp predation, but the mother is not able to move quickly enough to protect young more than 2.4 body lengths away (Cocroft 2002). Female wasps, *Gonizus nephantidis*, that lay eggs on caterpillars after paralyzing them, protect their offspring from conspecific intruders that are looking to use the same host (Goubault et al. 2007). *Copris incertus* is a beetle that broods its eggs in a nest chamber excavated from dung; each egg develops in a brood ball within the nest chamber (Halffter et al. 1996). The female cares for and maintains the brood balls and prevents desiccation of developing larvae as well as fungal invasion into the nest chambers and brood balls (Halffter et al. 1996). Maternal care in insects can take various forms depending on the threat to the offspring; therefore, it is important to understand the biology of a species before investigating it potential maternal care.

Peracarid crustaceans (amphipods, isopods, tanaids, and cumaceans) brood their young and sometimes exhibit maternal care. Most reproductive biology and behavior

studies on amphipods have, however, focused on the Gammaridea. Male gammarid amphipods can distinguish among different reproductive states of females upon contact and a series of investigative behaviors, after which non-receptive females and other males are ignored (Borowsky and Borowsky 1987). Once a male finds a receptive female, he guards her from other searching males until she molts, after which copulation takes place (Birkhead and Pringle 1985; Borowsky and Borowsky 1987). During copulation, the gammarid amphipod male grasps the female with his gnathopods and fertilizes the eggs as the female deposits them into the brood pouch. The brood pouch, which is external to the body, is formed by flat extensions called oostegites that extend medially from some thoracic coxae (Dunham et al. 1986). Embryos develop into juveniles within the protective brood pouch. Upon emergence, juveniles typically stay close to the parental female for an extended period of time before dispersing. Various maternal care studies have been performed on gammarid amphipods and show that the increased juvenile survival in the presence of maternal care outweighs the costs to the juveniles and parental females in these animals (Thiel 1998; Thiel 1999; Kobayashi et al. 2002).

Some species of caprellid amphipods also provide maternal care. Aoki (1997) observed newly emerged juvenile *Caprella subinermis* clinging directly to their mother, but they would disperse readily if disturbed. In the same study, *C. monoceros*, another species with juveniles that cling directly to the mother, showed maternal care (grooming and defending) for up to twenty days while juveniles grew and molted. Thiel (1997a) observed two females of *Aeginina longicornis* with juveniles attached; these juveniles were of two different size classes, suggesting they also grow while attached to their

mother. In this study, Thiel (1997a,b) suggested that, in addition protecting them from predation by fighting other caprellids, the mother increases the juveniles' access to food by lifting them higher into the water column where they can filter feed more effectively.

Our understanding that maternal care in caprellids can serve to protect offspring from other caprellids provides a foundation for several questions: what species and gender of adult caprellids presents the greatest threat to juvenile caprellids, and do conspecific caprellids cause mortalities of adults and juveniles? Maestripieri (1992) noted that where maternal aggression is present, offspring must be at risk from infanticide. Also, the maternal aggression shown in these cases lowers the risk of offspring mortality. Maestripieri suggested that conspecifics may be the source of the risk since juveniles create competition for resources, but the threat to caprellid juveniles is unknown. It would be energetically costly for a caprellid female to protect her young from all other caprellids if most conspecifics did not pose a threat. The ability to distinguish between predators and non-predators would be advantageous. Females could fight those species that pose a threat and ignore those that pose no threat, as seen in the skink *Mabuya longicaudata* discussed above (Huang 2006).

Other caprellids, especially conspecific males, may benefit from cannibalizing juveniles as is often found in wild felines (Natoli 1990). Cubs will often disappear from a pride of lions recently usurped by other males; this can force females to become receptive and produce the new males' cubs earlier and increase the reproductive success of his offspring by eliminating extra competition (Bertram 1975; Wolff 1985). In the caprellid, *Caprella monoceros*, females with newly-emerged juveniles removed can produce another brood almost 4 weeks earlier than if juveniles remained under her care (Aoki and

Kikuchi 1991). With this, male caprellids may be a threat to juveniles, as they are in wild felines, and directly benefit through their own reproductive success. Due to the extensive maternal care in these animals, females are not able to produce as many broods or offspring as related animals that do not provide this care. The reduction in female matings may correlate with a lower amount of mating opportunities for males. This could lead to high competition between males for receptive females, therefore, male-male aggression may also be prevalent in these species.

Mate choice and male-male aggression both control the outcome of sexual selection. Darwin (1871, as cited in Borgia 1979) also noted that species with sexual dimorphism also had highly variable male reproductive success, suggesting the presence of a polygamous mating system. In many polygamous systems, we see high male-male aggression, but even if female mate choice is involved, females can assess male abilities through fights, especially if the defeated male flees or dies (Borgia 1979). In species with male-male aggression, these competitions likely determine a male's reproductive success.

Male-male aggression has been reported in several caprellid species (Lewbel 1978; Lim and Alexander 1986; Caine 1991; Schulz and Alexander 2001). Lim and Alexander (1986) tested survival in male caprellids under three conditions: groups of males alone, groups of males with ovigerous females, and groups of males with receptive females. Though they define a receptive female as "about to molt," they do not state how they determined the female's stage in the molt cycle. I can only assume they used females without eggs or juveniles in the brood chamber. With only two trials of each treatment, survival of males with receptive females was significantly lower than the treatment containing only males (Lim and Alexander 1986). Aggression between males

also has been observed in *Caprella laeviuscula* when in the presence of females about to molt (Caine 1979). These studies and observations provide evidence for the ability of caprellid amphipods to identify receptive females and competitors for mating.

Maternal care and male-male aggressive behaviors could be important in caprellids for determining reproductive success at the juvenile and precopula stages of their life cycle. Juveniles may never reach reproductive maturity and males that lose male-male competitions may not attain a mate. When caprellids fight, they hit each other with their second gnathopods. Specialized parts, such as the poison tooth, associated with this appendage may play a role in the outcome of male-male aggressive encounters. Males may use this appendage to hurt or kill their opponents, therefore lessening their competition for mates. If males with poison teeth are more likely to mate with receptive females, then this could provide an example of sexual selection in *C. mutica*.

In the study presented in Chapter II, I ask the following questions of maternal care and male-male aggression: Is maternal care present in *C. mutica*? If so, from whom do the females protect their offspring? And does the presence of a non-brooding female increase the aggression between two males? To answer the questions of maternal care, I compare aggression levels of females of *Caprella mutica* in three reproductive states: a) with newly emerged juveniles, b) brooding offspring, and c) non-brooding, against conspecific caprellids of both sexes and against both sexes of a different caprellid species (*C. drepanochir*). These stimulus caprellids were added to the containers where the females had been isolated, and the number of attacks from the female was quantified. To address the issue of increased male-male aggression in the presence of a non-brooding female, two similarly sized males were placed together in a small container, and a female

of one of the reproductive states or a male was introduced. Fights between the two original males were quantified and compared to the other treatments including the control of no stimulus caprellid.

In Chapter III, I ask the question: Does the presence of poison teeth increase mortality in aggressive encounters between males? To test this question, I placed two males, one with poison teeth and one with poison teeth experimentally removed, with a non-brooding female for one day. Survival rates were recorded and compared among treatments to determine if poison teeth increase mortality in aggressive encounters between males.

CHAPTER II

MATERNAL CARE AND MALE-MALE AGGRESSION IN THE CAPRELLID AMPHIPOD, *CAPRELLA MUTICA*

INTRODUCTION

Caprellid amphipods are benthic animals associated with a variety of living substrata. They have been recorded on algae, seagrasses, sponges, gorgonians, sea anemones, scyphozoans, hydroids, large crustaceans, mollusks, echinoderms, bryozoans, and sea turtles (Guerra-Garcia 2001; Thiel et al. 2003; Willis et al. 2004). Species composition of caprellids within a habitat varies with the hydrodynamics and depth of the substratum (Guerra-Garcia 2001; Thiel et al. 2003). Caprellids cling to substrata mostly with their posterior appendages (pereopods), but can also use their gnathopods both to cling and for locomotion. Species can be divided into two groups based on their clinging posture: parallel—clinging with pereopods and gnathopods, and upright—clinging mostly with pereopods (Takeuchi and Hirano 1991, 1995). Upright species are usually filter-feeders found in areas with little wave action, whereas the parallel species commonly scrape and consume the substratum and are most prominent in areas with strong wave action (Takeuchi and Hirano 1995).

Within the Amphipoda, reproductive biology and behavior are studied most commonly in the suborder Gammaridea. Male gammarid amphipods can distinguish among different reproductive states of females and are known to guard receptive females before they molt, after which copulation takes place (Birkhead and Pringle 1985; Borowsky and Borowsky 1987). During copulation, the gammarid amphipod male grasps the female with his gnathopods. The eggs are fertilized externally when deposited into the brood pouch, which is formed by flat extensions, oostegites, that extend medially from some thoracic coxae (Dunham et al. 1986). The embryos develop into juveniles that emerge from the brood pouch and typically are cared for by the parental female for an extended period of time before dispersing. Within gammarids, maternal care has been observed in many species. Female individuals of *Casco bigelowi* care for their young in a burrow until they are more than half the adult size (Thiel 1998). A study of costs and benefits of maternal care in another gammarid amphipod, *Parallorchestes ochotensis*, revealed that the benefits of protecting offspring from predation outweigh the cost of their suppressed growth in the presence of the parental female (Kobayashi et al. 2002). The general benefit of maternal care is that young are raised to a larger size, which may discourage predation and aid in competition when resources are limited (Thiel 1999).

Reproductive biology and behavior in the suborder Caprellidea is less studied, but similar in many respects to that of gammarids. In contrast to gammarid amphipods, male caprellids grasp the females using their pereopods and assist her in molting using their first gnathopods before mating occurs (Caine 1991). Published data are lacking on where fertilization takes place in caprellids. Female caprellids develop a brood pouch on pereonites 3 and 4. The brood pouch is made of four interlocking flaps, called oostegites,

which extend medially from the pereonites and arc around to form a chamber on the female's ventral side. Females aerate the eggs during development by moving oostegites, and emerged juveniles often spend an extended amount of time on or near the parental female before dispersing. As observations of amphipod reproductive biology can generate questions of the presence of maternal care and male-male competition for mates, I recorded prominent behaviors associated with reproduction.

Some species of caprellids exhibit maternal care, but these behaviors have not been described in detail or investigated experimentally (Lim and Alexander 1986; Aoki and Kikuchi 1991; Aoki 1999). Juveniles, newly-emerged from the brood pouch, cling either directly to the female or to the substratum around her. The juveniles' proximity to the mother and the duration of maternal care are variable and depend on the species. Aoki (1999) reports *Caprella arimotoi*, *C. danilevskii*, *C. glabra*, *C. okadai*, and *C. penantis* show no maternal care, while juveniles of *C. scaura*, *C. monoceros*, and *Pseudoprotella phasma* cling directly to the mother, and juveniles of *C. decipiens* cling to the substratum around the mother (Aoki and Kikuchi 1991). Small fish are known predators of caprellids (Caine 1989), but it is possible that other caprellids may consume or pose other threats to juveniles. Maternal care in caprellids may protect juveniles from hostile caprellids, but the sex and species of caprellids that pose a threat to juveniles is unknown.

Benefits of maternal care are best understood in birds and mammals, but concepts developed in these studies may apply to invertebrates as well. Infanticide is common in birds (Pierotti and Murphy 1987; Pierotti 1991), as mothers kill foreign young that, through adoption, will draw resources from their own young, and in mammals, where males slay nursing juveniles to mate with their mothers earlier than if natural weening

took place (Ebensperger 1998). *Caprella scaura typica* exhibits maternal care behavior, described vaguely as “fighting” or “fleeing with the juveniles” when threatened by other caprellid or gammarid amphipods (Lim and Alexander 1986). The same behavior was seen in *C. decipiens* when they were disturbed by shaking the container (Aoki and Kikuchi 1991). The variation in maternal care and in behaviors that may be the result of maternal care within caprellids provide a unique opportunity to experimentally test the hypotheses about maternal care that were developed in studies of vertebrates: parental females protect juveniles from conspecific females, who may be avoiding adoption of these unrelated juveniles, and from conspecific males, who would mate with the female earlier if juveniles were killed.

The proximity of juveniles to their mother and the time spent within her care may come with a cost to the female. Juveniles of *C. monoceros* stay close to their mother, and she will not molt for 30 days or more when juveniles remain under her care (Aoki and Kikuchi 1991). When juveniles were removed, females of this species were able to produce another brood within three days (Aoki and Kikuchi 1991). This suggests that if juveniles dispersed immediately after emergence, the female could produce more young. Additionally, caprellid species that show less maternal care usually produce another brood earlier than caprellid species that show more extensive maternal care (Aoki and Kikuchi 1991; Thiel 1997b). A mother who provides more care to her juveniles may pay the cost of not producing as many offspring, however, may benefit by increasing the survivorship of her brood. Knowledge of maternal care in caprellid amphipods is limited to the few species mentioned in the studies above.

An extended period of maternal care may limit mating opportunities for males and, therefore, cause aggression between them as they fight for mates. Females who provide extended maternal care may have a reduced mating frequency than those who do not provide maternal care, resulting in a lower number of receptive females at any given time. For females who do not provide maternal care, the higher frequency in females mating provides more opportunities for males to find a mate. Therefore, in the presence of extended maternal care, mating opportunities for males decrease and competitions between males for mates can increase. Male aggression has been observed in macaques, where females mate with males that partake in aggressive encounters with other groups (Cooper et al. 2004). This is an example of female choice resulting in high male aggression between groups. In mountain goats, heavier males are likely to win more male-male competitions and spend more time courting females (Mainguy and Cote 2008). This is an illustration of male-male aggression where males fight for mating opportunities, which can result in sexual selection favoring heavier males.

Male-male aggression in caprellids has been seen in the presence of receptive females and occasionally results in the death of a male (Caine 1980; Lim and Alexander 1986; Schulz and Alexander 2001). Lim and Alexander (1986) reported that approximately 50% of males in the presence of receptive females survive whereas nearly all males survive when with males or ovigerous females. A protrusion, historically called the "poison tooth", is located on the palmar surface of the second gnathopods of males in the genera *Caprella*, *Paracaprella*, *Luconacia*, *Paradicaprella*, and *Aciconula* (Schulz and Alexander 2001). The poison tooth is often cited as the reason for fatalities during male-male interactions, but there has not been sufficient work done on the poison tooth in

order to determine if it causes fatal injuries (Lim and Alexander 1986; Caine 1991; Schulz and Alexander 2001).

The avoidance of costs in raising unrelated young and costs in the protection of offspring from infanticidal individuals produce aggressive behaviors in female vertebrates. Limited access to mates during brooding also produces aggressive behaviors in male vertebrates. Since evolutionary pressures may act upon brood care similarly in all animals, the costs and benefits of maternal care in caprellid amphipods may produce the same behaviors observed in vertebrates. This study compares aggression levels in caprellid females with newly emerged juveniles, females brooding embryos, and non-brooding females. Aggression toward male and female conspecifics and toward male and female congenerics was measured for females in all three reproductive states to examine possible parallels to the existing models of maternal care in vertebrates and amphipods.

Caprella mutica Schurin, 1935 is the study species and the congeneric species included in this study is *C. drepanochir* Mayer, 1890. I hypothesize that parental females of *C. mutica* will attack other caprellids regardless of sex and species, as has been seen in C. *monoceros* (Aoki and Kikuchi 1991). Additionally, I hypothesize that aggression in parental females will be higher than aggression in non-brooding and ovigerous females.

This study also examines male-male aggression in *C. mutica* when a female in one of the three reproductive states or a male is introduced to a container holding two males. I hypothesize that males will show the most aggression toward other males in the presence of a non-brooding female and less aggression when in the presence of other types of females or other males. In this experiment, I pose the following questions: Are there differences in male aggression in the presence of non-brooding females compared

to male aggression in the presence of females in other reproductive states, another male, and males alone? And is male aggression different in the presence of females in all three reproductive states compared to male aggression when left alone and in the presence of just another male? *C. mutica* was an ideal species with which to test my hypotheses, as large populations were readily available during the summer months and are easily manipulated in the lab.

MATERIALS AND METHODS

Caprellid collection and maintenance

Caprella mutica were collected from red filamentous algae in the small boat basin, Charleston, Oregon (Figure 1). Large numbers of caprellids were kept with red filamentous algae in large finger bowls in a seatable with flowing seawater and an airstone, to ensure high oxygen concentrations. Keith (1969) reports that caprellids (*Caprella* spp.) are omnivores, but Takeuchi and Hirano (1995), in their posture study, list some species as primarily filter-feeders and others as 'scrapers'. *C. mutica* spent most of its time in the upright position, so I assumed it was filter-feeding. However, they readily accepted and devoured bits of mussel. Caprellids given filamentous red algae with healthy epiphytic diatom communities were more active and survived longer than those given mussel tissue. Epiphytic diatoms have been seen to grow on caprellids themselves, and *C. mutica* has been observed grooming, possibly consuming growing epiphytes (Figure 2). Caprellids kept for long periods were given fresh red filamentous algae approximately once per week.

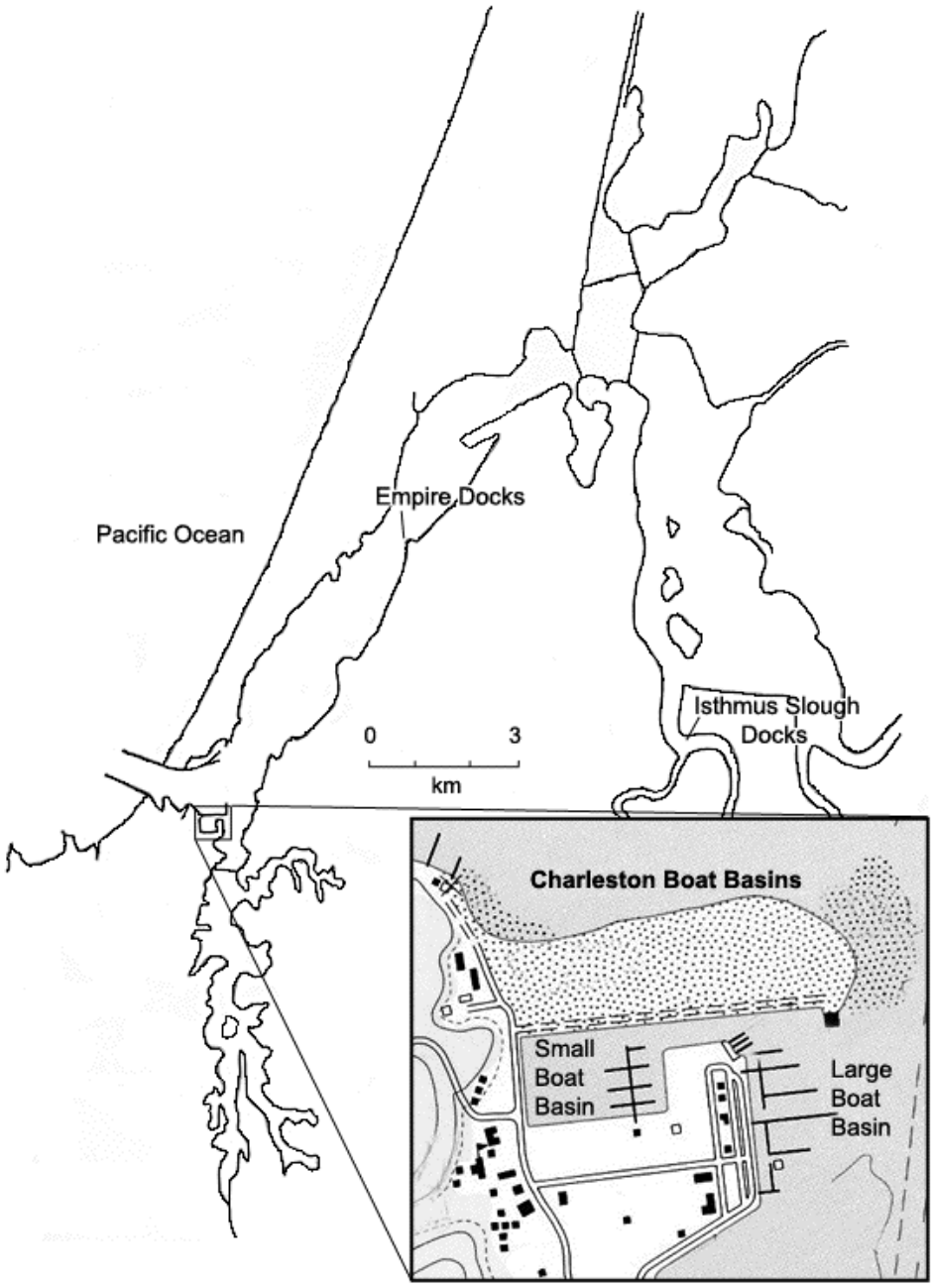

Figure 1: Line drawing of Coos Bay and enlargement of the boat basins in Charleston, Oregon. Traveling upriver in Coos Bay, the Empire Docks are located approximately halfway between the mouth and the north end of the bay. Isthmus Slough is located at the Southeast arm of the bay. The boat basins in Charleston are just beyond the inlet of the bay. The small boat basin in Charleston is more enclosed, connected to the large boat basin by a small channel. The large boat basin is more exposed, as it sits directly off the channel that extends south from Coos Bay into South Slough. Line drawing adopted from Hewitt 1993, enlargement of boat basins from USGS 1970.

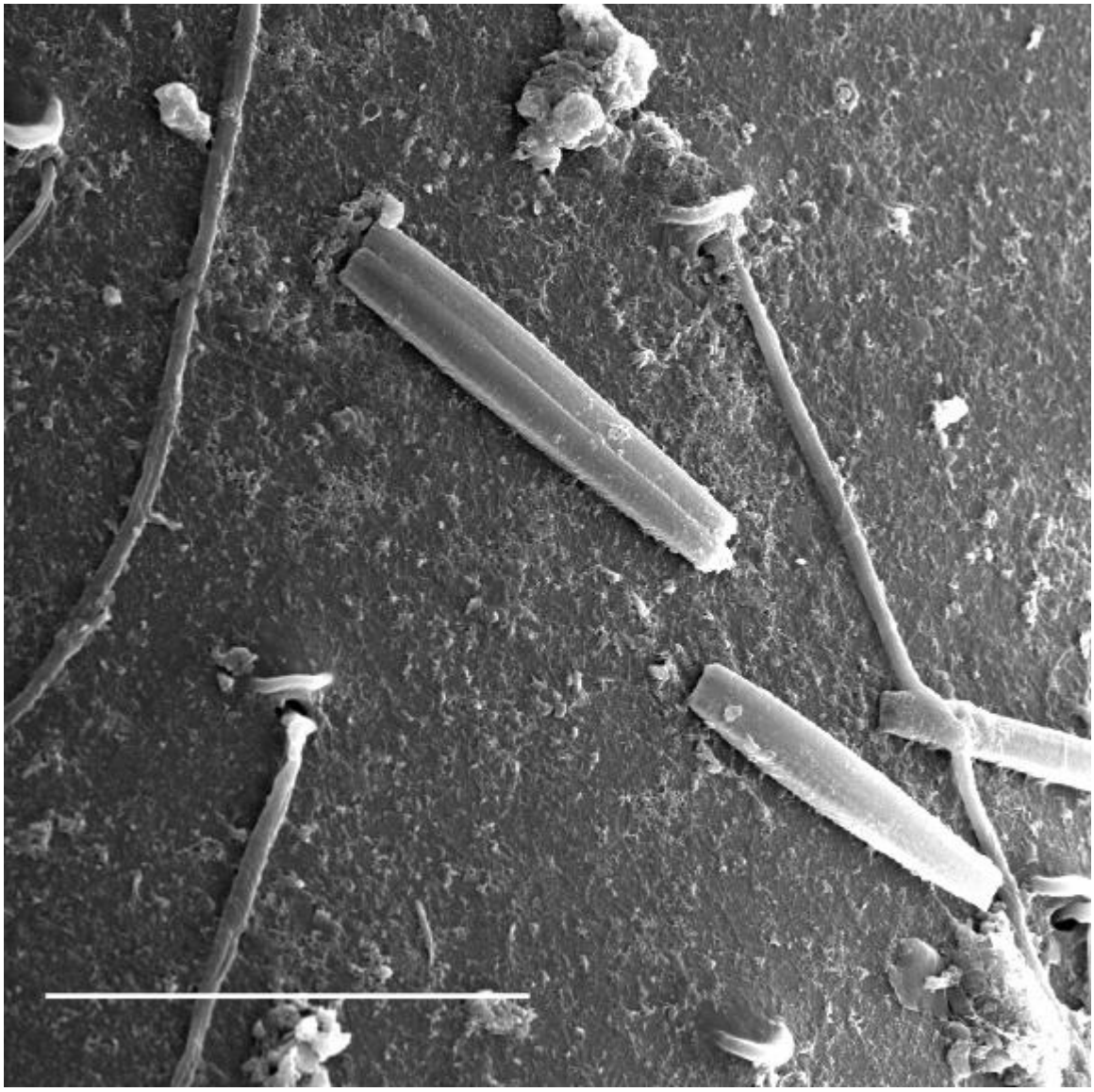

Figure 2: SEM micrograph of diatoms located on second gnathopods of a male *Caprella mutica*. Scale bar measures 50 μm.

Females were isolated in Toby Teaboys (Aldridge Plastics, Aldridge, UK; 150-μm mesh netting) with a small amount of algae and kept in a seatable with flowing seawater and an airstone. Ovigerous females designated to be tested as parental females were held individually in Toby Teaboys until juveniles emerged. Behaviors of parental

females were observed within 24 hours of juveniles emerging. Due to varying amounts of maternal care (Aoki 1997) and my personal observations with *C. mutica*, I chose to test parental females in the first 24 hours to increase the likelihood that I was testing maternal care behavior before juveniles attempt to disperse. Females in Toby Teaboys were monitored daily.

C. drepanochir, used in the maternal aggression experiment, were collected from the hydroid, *Obelia*, growing on Vexar ® netting attached to the docks in the large boat basin, Charleston, Oregon (Figure 1). They were maintained in a large finger bowl in a seatable in the same manner as *C. mutica*.

Maternal care

Females of *Caprella mutica* were isolated in Toby Teaboys for at least 24 hours before testing. For each trial, the Toby Teaboy was opened, and the half containing the caprellid female and algae was set in a small bowl of seawater. Stimulus caprellids (four treatments described below) were introduced for ten minutes. The trial time of ten minutes was determined in a pilot study. In the pilot study, two trials for each treatment were recorded on videotape for 20 minutes using a black and white camera elevated on a tripod. Trials were watched on a monitor, and the number of attacks was counted along with the time of each attack after the stimulus caprellid had been introduced. Data from the pilot study showed that the majority of fights occurred within the first 10 minutes of each trial. To determine whether parental females would fight stimulus caprellids and could distinguish which stimulus caprellid posed the greatest threat to the juveniles, I counted how many full attacks were directed at each stimulus caprellid using the same video set-up as described in the pilot study. A full attack or fight was defined as the

tested female hitting the stimulus caprellid with both second gnathopods (Schulz and Alexander 2001). Full attacks also resulted in the stimulus caprellid fighting back or fleeing. Each treatment was described by two factors for predicting female aggression: female reproductive state (non-brooding, ovigerous, or parental) and stimulus caprellids (conspecific females, conspecific males, congeneric (*C. drepanochir*) females, and congeneric (*C. drepanochir*) males). There were 10 replicate trials for each treatment, each testing aggression by a different female. For treatments with female stimulus caprellids, non-brooding females and ovigerous females were used in 5 trials each.

For statistical analysis, the number of full attacks per trial was changed into binary (presence/absence) values for thirty individual tests of logistic regression with dummy coding for categorical variables. There is an increase in Type I error rate with this many repeated tests. I acknowledge that the probability for Type I errors is dramatically increased, however, some would argue that using the sequential Bonferroni analysis to correct this would increase the probability of Type II errors (Nakagawa 2004). Therefore, I did not correct any of the p-values given from the logistic regression tests. Logistic regression was used to compare aggression of non-brooding and ovigerous females to that of parental females. The percentage of females that fought were combined for each reproductive state and compared using a goodness-of-fit G-test. Since G-tests are prone to Type I errors, the calculated statistic was adjusted by Williams correction factor (Williams 1976; Sokal and Rohlf 1987). This transformation was performed for both goodness-of-fit tests in this study. Additionally, the number of full attacks per female was combined for each reproductive state for comparison using the X^2 test.

Lastly, average fights in 10 minutes were compared across all treatments using a two-way ANOVA with female reproductive state and stimulus caprellid as factors.

Male-male aggression

To test whether behavior of males of *Caprella mutica* changes in the presence of females in various reproductive states or other males, pairings were set up in the lab and aggressive behavior was quantified. For male-male interactions, two males were placed in a 35x35-mm container. Males in each treatment were similarly sized (mean difference each trial ± SE: 1.1 ± 0.1 mm, n=80; mean length, cephalon to abdomen, for all trials ± SE: 12.6 ± 0.2 mm, n=176). These two males were allowed 20 minutes to acclimate to the container. After acclimation, a stimulus caprellid was added and attacks between the two original males were recorded for 10 minutes (Figure 3). The trial time of 10 minutes was chosen after a pilot study. In the pilot study, one trial from each treatment was recorded for 20 minutes using the same video set-up as described in the previous pilot study, and the majority of fights again occurred during the first 10 minutes after the stimulus caprellid was introduced. Stimulus caprellid treatments included: non-brooding females, ovigerous females, parental females with juveniles, and a conspecific male. The control treatment was observations of the two males without a stimulus caprellid. There were 16 replicate trials for each treatment. Non-brooding females were used to represent receptive females. Previously, receptive females have been defined as "about to molt" (Caine 1979; Lim and Alexander 1986). To investigate whether male aggression was influenced by the maturity of eggs, I measured oocyte size for each non-brooding female before each trial. Developing oocytes were measured with a compound microscope by viewing the ovary through the female's dorsal side.

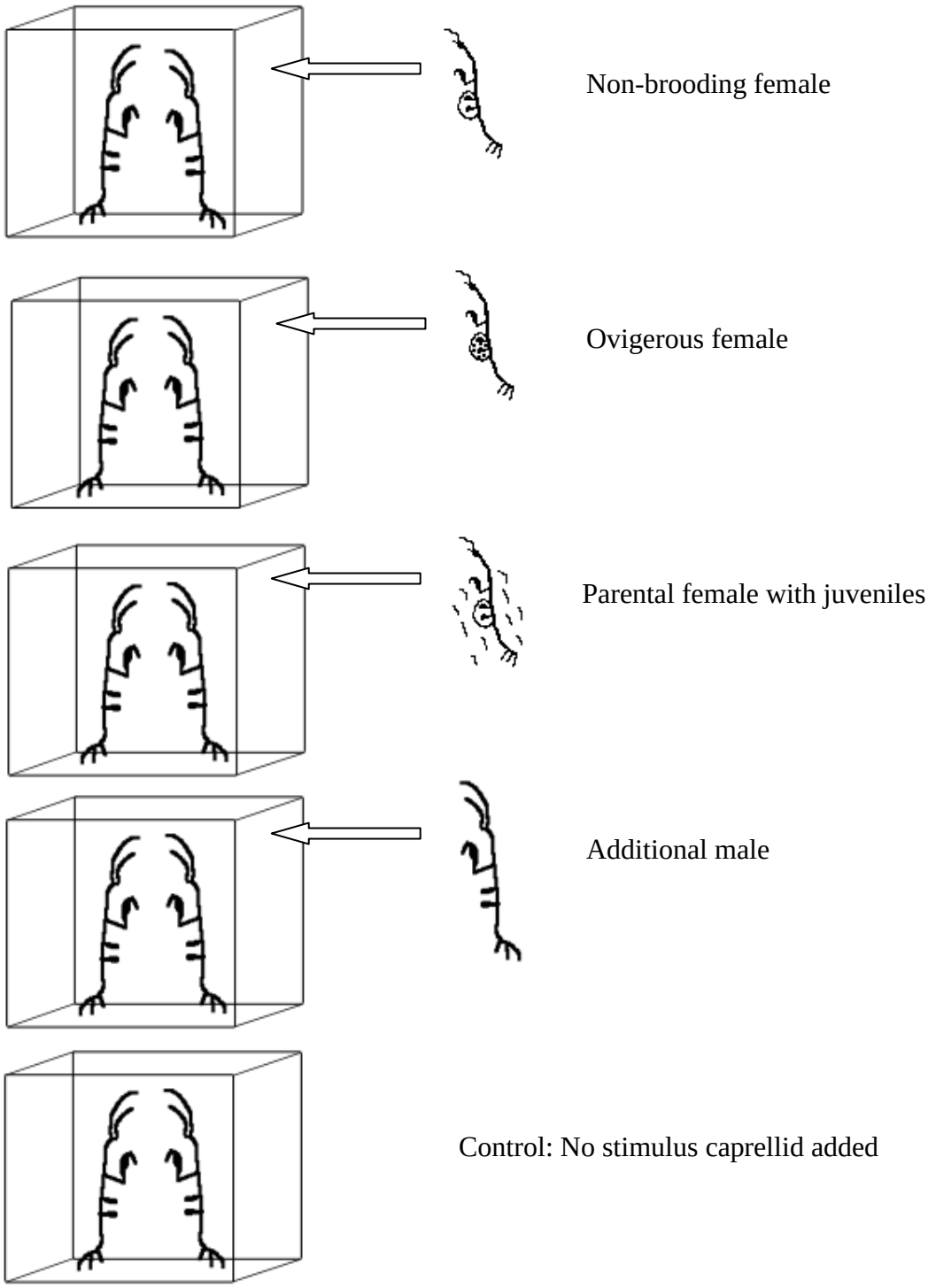

Figure 3: Experimental set-up of male-male aggression experiment. Two males were isolated for 20 minutes before a stimulus caprellid was added. Fights between the two original males were counted for 10 minutes after 20-minute acclimation time.

The number of full attacks between the two original males was recorded for each 10-minute trial. For statistical analysis, these data were changed into categorical fight/no fight values and compared using ten individual tests of logistic regression with dummy coding for categorical variables. P-values were not corrected using the sequential Bonferroni analysis (see explanation above). Data from different treatments were pooled to answer questions posed above. The percentage of males that fought in each treatment was compared using a goodness-of-fit G-test. The mean number of fights per trial was compared with ANOVA. A Pearson Correlation was used to determine if the number of fights between males covaried with developing oocyte size in non-brooding females.

RESULTS

Observations of occurrence, reproduction, and juvenile care

Caprella mutica occur in high abundances on the floating docks in the small boat basin, Charleston, Oregon from late spring through the fall. They are also found further upriver in Coos Bay in the summer on the floating docks in Empire and Isthmus Slough (Figure 1). In the Charleston Boat Basin, abundances of *C. mutica* decrease in the winter, but it is unknown whether they move to deeper water or a large portion of the population dies.

While monitoring caprellids for lab experiments, I observed many behaviors associated with the reproductive biology of *C. mutica* that had not been noted previously. Although only seen twice, amplexus lasted 10-15 minutes. Each time, immediately after the male departed, the female deposited eggs into her brood pouch. In oviposition, she bends so that pereonites 4 and 5 form a 90-degree angle. This aligns her genital opening,

located on the ventral side of pereonite 5 with a gap in the brood pouch, which extends from pereonites 3 and 4. It is unclear where sperm were located or how the eggs were fertilized. It is unlikely that females of *C. mutica* store sperm. After producing a successful brood, a female of *C. mutica* would sometimes extrude another group of eggs into her brood pouch, but this second brood never developed into juveniles. If *C. mutica* could store sperm, these secondary broods might be fertilized, and females could produce more juveniles.

Within 24 hours of juveniles emerging from the marsupium, there could be anywhere between one to fifty juveniles surrounding the female. Some were seen clinging directly to the female, although most were on the substratum around her. When parental females of *C. mutica* were kept in a solid container with an open top and flowing seawater, most of the juveniles dispersed within 7 days. Although measurements were not taken, when maintained in a closed system like the Toby Teaboy, the distance between mother and juveniles would increase after one day. Therefore, maternal care, if any, in *C. mutica* is short-lived.

Maternal care

Pairwise comparisons were made amongst females in different reproductive states for each type of stimulus caprellid (12 paired comparisons; see Table 1). Comparisons within similar stimulus caprellids were all non-significant except: a) parental female and non-brooding female attacks on males of *Caprella drepanochir*; and b) parental female and ovigerous female attacks on males of *C. drepanochir*. In both of these comparisons, the number of parental females attacking males of *C. drepanochir* was higher. Pairwise comparisons were also made within each reproductive state across all types of stimulus

caprellids (18 paired comparisons). Comparisons within similar female reproductive states were all non-significant except that the number of parental females attacking males of *C. drepanochir* was significantly greater than the number of parental females attacking females of *C. mutica*.

Table 1: P-values from individual logistic regressions performed on treatments in the maternal care experiments. Non-brooding (N), Ovigerous (O), and Parental (P) indicate the reproductive state of the tested female. Stimulus caprellids include conspecific females (Cm F) and males (Cm M) and congeneric females (Cd F) and males (Cd M). P-values in the two diagonals located toward the upper right-hand corner of the table are comparisons made amongst females in different reproductive states, but with the same type of stimulus caprellid. The other three blocks of p-values are comparisons made amongst types of stimulus caprellids tested with females of the same reproductive state. Bold values indicate significant difference between treatments.

		Non-brooding			Ovigerous				Parental			
		Cm F	Cm M	Cd F	Cm F	Cm M	Cd F	Cd M	Cm F	Cm M	Cd F	Cd M
N	Cm F				0.982				0.982			
	Cm M	0.979				0.640				0.640		
	Cd F	0.981	1.000				1.000				1.000	
	Cd M	0.982	0.284	0.185				1.000				**0.035**
O	Cm F								1.000			
	Cm M				0.147					1.000		
	Cd F				0.284	0.640					1.000	
	Cd M				1.000	0.147	0.284					**0.035**
P	Cm M								0.147			
	Cd F								0.284	0.640		
	Cd M								**0.035**	0.374	0.185	

To test my hypothesis that parental females are more aggressive than non-brooding and ovigerous females, data from non-brooding and ovigerous females were pooled and compared with that of parental females using logistic regression. I avoided pooling data with significant differences by splitting these comparisons by type of stimulus caprellid. Although these pooled data within each type of stimulus caprellid may have differences I could not detect using statistics, I pooled them to ask a specific

question and did not combine any treatments that were significantly different in previous comparisons of logistic regression. None of these pairwise comparisons was significant (Table 2).

Table 2: P-values from logistic regressions of pairwise comparisons of attacks by parental females with attacks for non-brooding and ovigerous females combined within each type of stimulus caprellid.

Stimulus category	
Conspecific Females	0.611
Conspecific Males	0.789
Congeneric Females	1.000
Congeneric Males	0.051

When comparing the percentage of females fighting all stimulus caprellids, aggression increased from non-brooding to ovigerous females and from ovigerous to parental females (Figure 4). The goodness-of-fit test for these percents is not significant ($G=2.5$; $p=0.52$). The same increasing trend is observed when comparing the mean number of fights per fighting female (Figure 5). These values are significantly different ($X^2=7.2$; $p=0.028$). Due to this trend, the number of parental females fighting is not significantly more than non-brooding and ovigerous females, but the parental females that are fighting are exhibiting a greater number of attacks on all stimulus caprellids than non-brooding and ovigerous females.

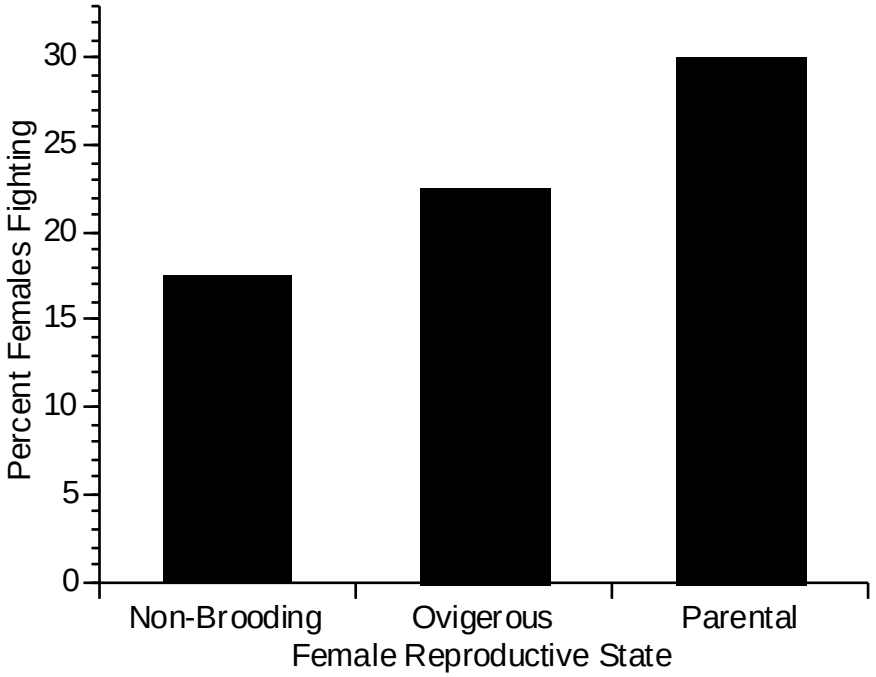

Figure 4: Percent females fighting from three female reproductive states against all stimulus caprellid types: both sexes from *Caprella mutica* and *Caprella drepanochir*. Percents are out of 40 females, divided equally amongst stimulus caprellid types. Across all stimulus caprellid types, there is no difference in percent population that would behave aggressively among reproductive states (G=2.5; p=0.29).

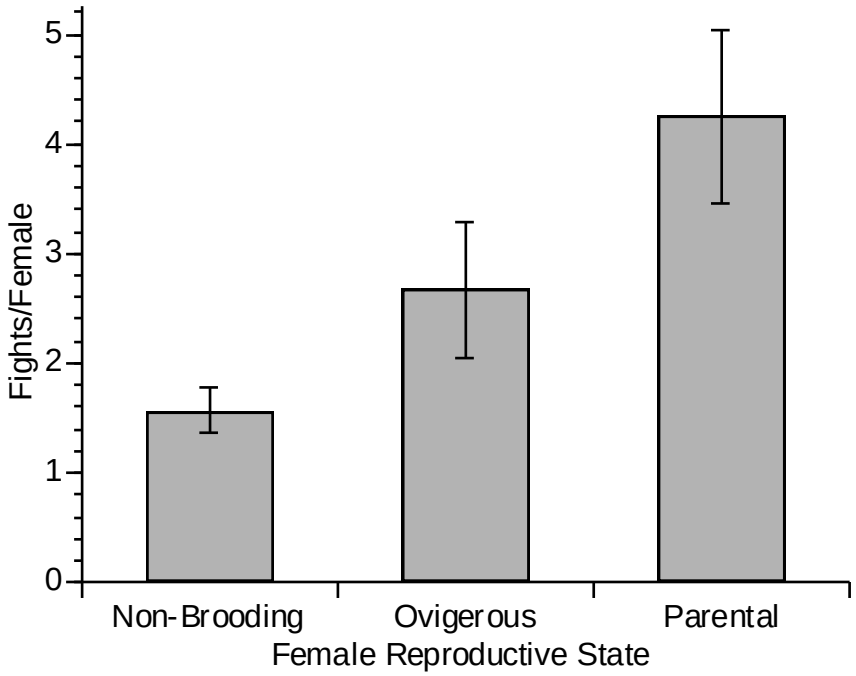

Figure 5: Mean number of fights per fighting female for each female reproductive state across all stimulus caprellids. Averages out of 7 for non-brooding, 9 for ovigerous, and 14 for parental females. Error bars represent one standard error. Values are significantly different (X^2=7.2; p=0.028).

When the total number of fights is characterized by treatment, there is very little aggression toward conspecific females (Figure 6). Aggression toward conspecific males increases with sequential reproductive levels. Females of *C. drepanochir* receive the most aggression from ovigerous females, with moderate attacks from the others, while males of *C. drepanochir* are attacked very few times from non-brooding and ovigerous females and receive an extremely high number of attacks from parental females. Given the equal sample sizes, a graph of mean attacks per trial would show the same patterns. The two-way ANOVA could not be performed on the average number of fights in 10 minutes because of violations in the assumptions of normality and homogeneous variances across treatments. Instead, I used the non-parametric Kruskal-Wallis test and

found that differences among the number of fights in these treatments were significant at p=0.058 (H=19.2).

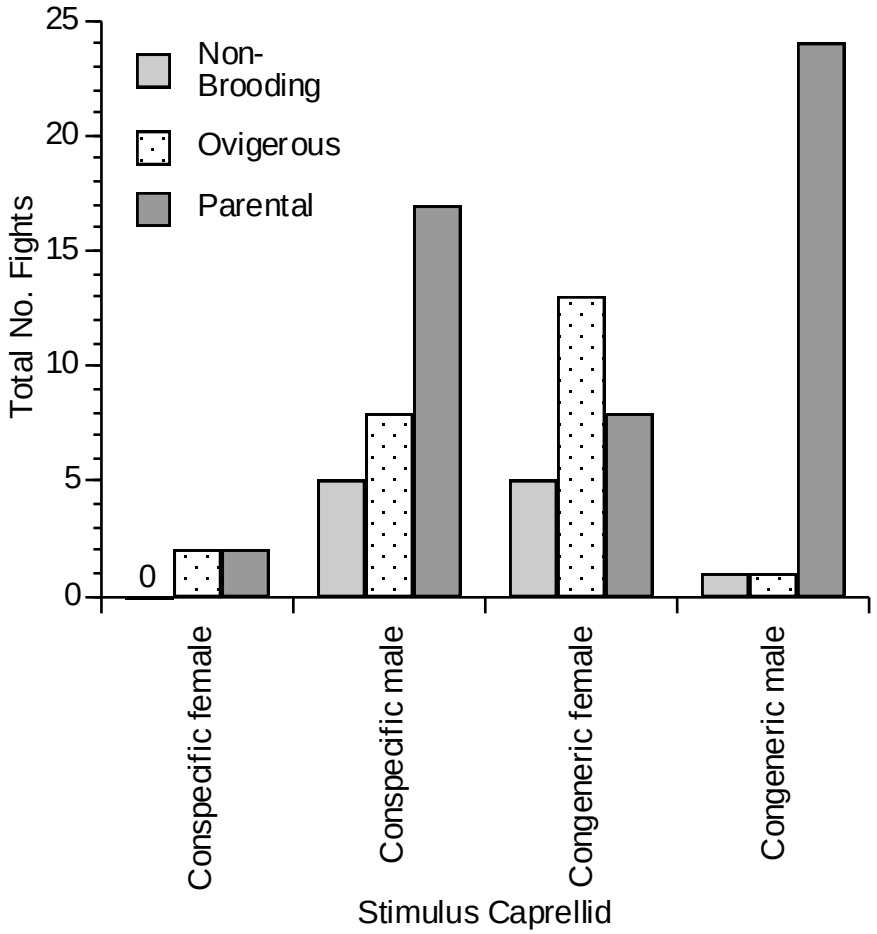

Figure 6: Total number of fights in ten minutes from all females, categorized by stimulus caprellid and female reproductive state. Bars represent total fights from 10 females. Parental females are more aggressive toward males than females and in general more aggressive than other female reproductive states. These values are significant at p=0.058 (H=19.2).

Male-male aggression

In male-male aggression trials, most attacks were between the two original males, with one male generally being the attacker. However, males were commonly seen fighting with females in the ovigerous and parental female treatments.

No significant differences in male-male aggression between treatments were found in pairwise comparisons, except when comparing male aggression in the presence of a non-brooding female to aggression in the presence of another male (Table 3). A greater number of males fought each other in the presence of a non-brooding female than in the presence of another male. Again, data from various treatments were pooled to answer previously posed questions, although pooled data may have differences not detected by statistics. First, the aggression between males was significantly higher with a non-brooding female stimulus caprellid than with all other treatments combined (t-ratio=2.2; p=0.029). Secondly, male aggression in treatments of female stimulus caprellids (non-brooding, ovigerous, and parental) was significantly higher than male aggression in treatments containing only mores at p=0.058 (t-ratio=1.9).

Table 3: P-values from individual logistic regression models comparing data from treatments of male-male aggression. Treatments include three types of female stimulus caprellids (non-brooding, ovigerous, and parental), a male stimulus caprellid, or no stimulus caprellid. Bold value indicates significant difference between male-male aggression in presence of non-brooding female and in the presence of an additional male.

	Non-brooding	Ovigerous	Parental	Male
Ovigerous	0.150			
Parental	0.284	0.695		
Male	**0.032**	0.373	0.213	
None	0.071	0.670	0.418	0.628

Males are most aggressive in the presence of females and this trend was seen additionally in the mean number of fights exhibited by males in these treatments (Figure 7). Although the number of fights for all female-stimulus treatments was higher than male and no stimulus caprellid, their differences among all treatments were not significant (F=1.14; p=0.34).

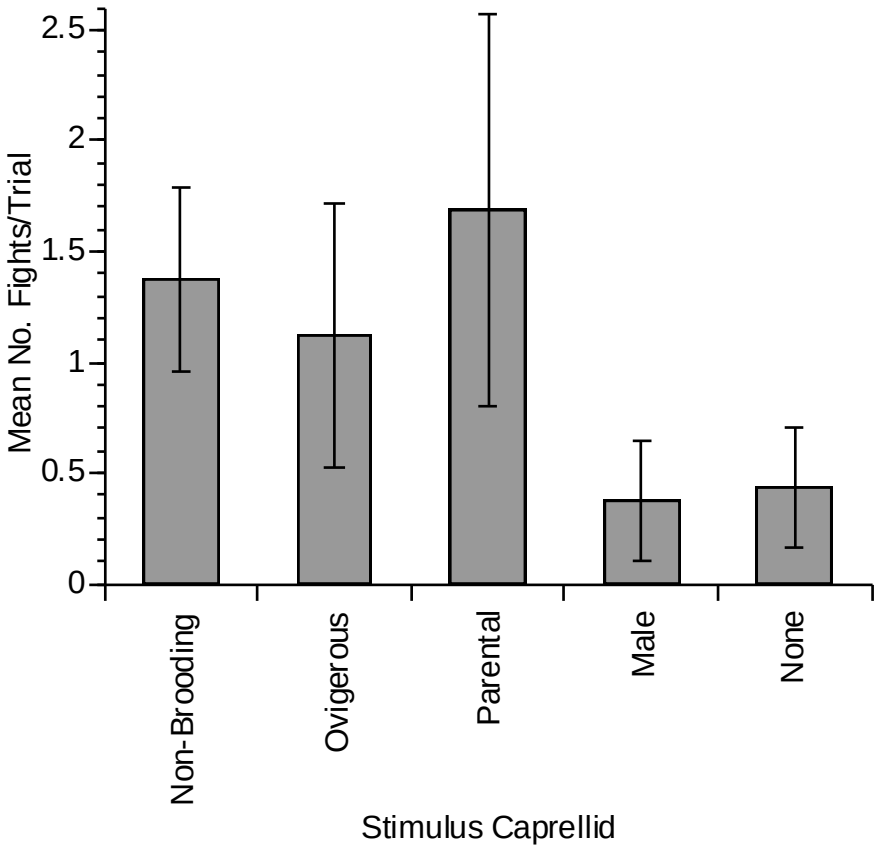

Figure 7: Mean fights in ten minutes between two males for each stimulus caprellid treatment. Error bars indicate one standard error. N=16 for each treatment. When comparing all treatments using ANOVA, these means are not significantly different ($F=1.14$; $p=0.34$).

As with mean number of fights per trial, the highest percentage of males fighting was in the female-stimulus treatments, but the goodness-of-fit test shows no significant difference between all treatments ($G=4.43$; $p=0.35$; Figure 8). The highest percent of males fighting was in the non-brooding female treatment, followed by the parental females.

When the number of attacks in 10 minutes are plotted against oocyte size, there are no attacks between males in the presence of females with small (< 150 μm) and large (> 275 μm) oocytes. For trials with females' oocytes measured between these values, the

number of attacks between males was extremely variable (Figure 9). The correlation between number of attacks between males and oocyte size of female present was non-significant (r=0.30; p=0.28).

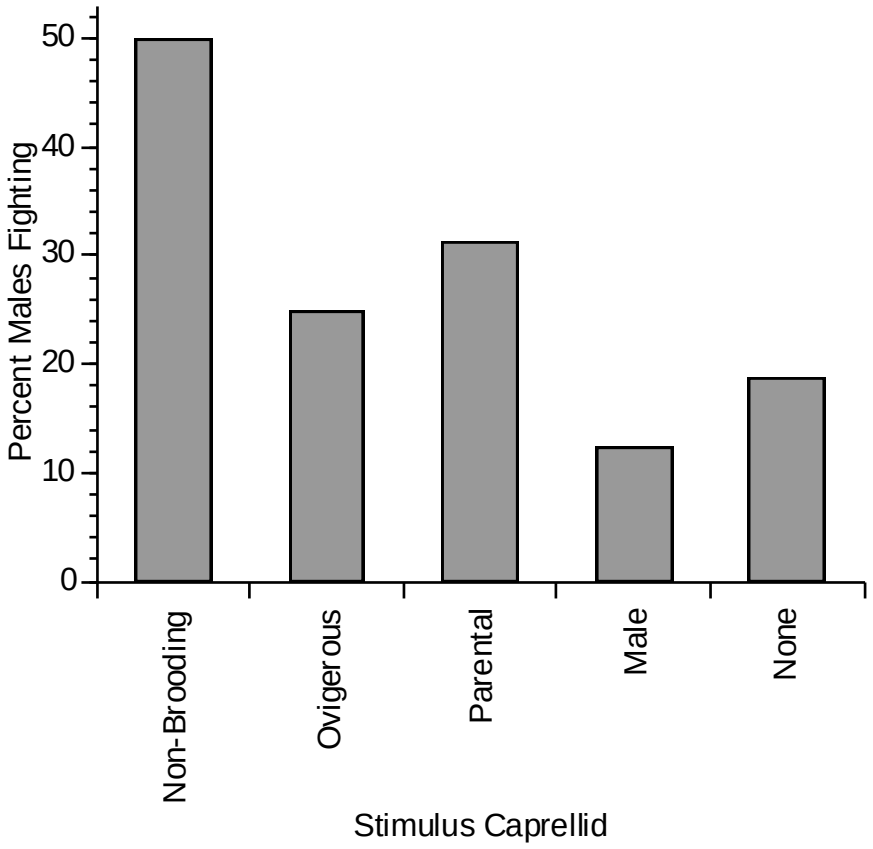

Figure 8: Percent males fighting in the presence of each stimulus caprellid. Percents are out of 16 pairs of males. Percentage of males fighting is highest in the presence of a non-brooding female, but differences are not significant (G=4.43; p=0.35).

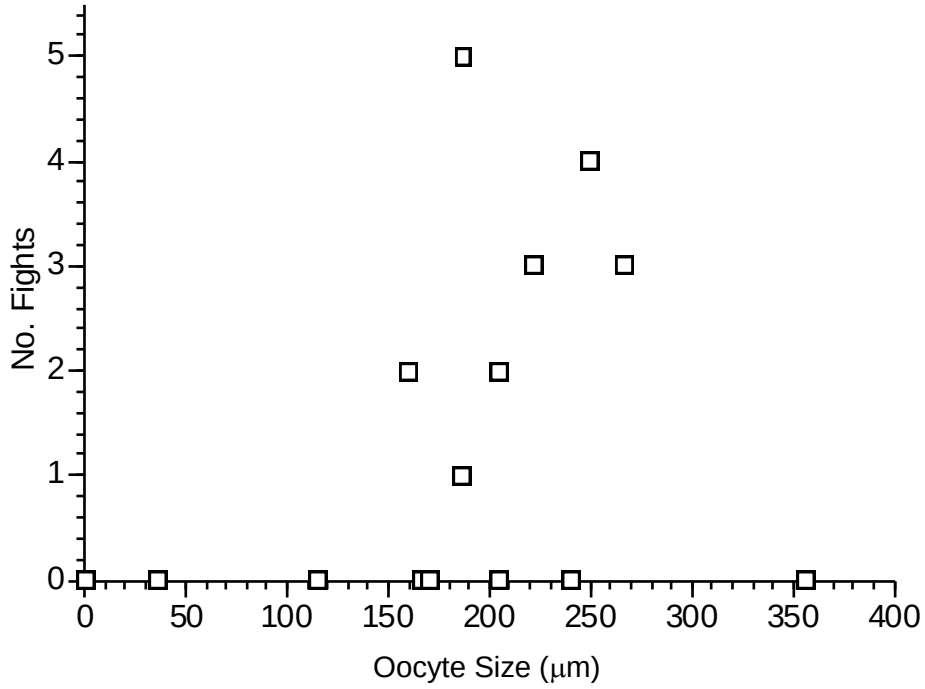

Figure 9: Number fights in ten minutes between males in the presence of non-brooding females by the corresponding female's oocyte size. Each of the 16 trials tested with different females. One oocyte size is missing. Correlation is not significant (r=0.30, p=0.28).

DISCUSSION

The number of parental females of *Caprella mutica* attacking males of *C. drepanochir* was significantly higher than the number attacking females of *C. mutica* and the number of other females attacking males of *C. drepanochir*. The total number of fights for each treatment also illustrates these large differences. This suggests that parental females recognize males of this species as a threat to their young. The threat congeneric males pose to juveniles is unknown. Possible explanations include that juveniles of *C. mutica* and congeneric males are competing for food or congeneric males eat juveniles of *C. mutica*. Since I rarely saw these two species on the same substratum or on two different

substrata in close proximity, it is unlikely they are competing for food. Also, in this case, one would expect that females of *C. drepanochir* would compete with juveniles for food; however, the high aggression from parental females of *C. mutica* toward males of *C. drepanochir* is not seen toward females of *C. drepanochir*, which suggests these females do not pose the same threat. If males of *C. drepanochir* eat juveniles of *C. mutica*, the most apparent adaptive purpose would be for nutritional value. I am unsure how much nutrition juveniles provide, but they may be easily captured in the absence of a parental female. However, no threatening behaviors, such as predation, were seen when congeneric males were placed in the same container as juveniles with parental females removed.

Additionally, the total number of parental female fights toward males of *C. mutica* is more than twice the total fights from ovigerous females and more than three times the total fights from non-brooding females on males of *C. mutica*. This suggests the possibility of conspecific males posing a threat to juveniles of *C. mutica*. When a male *C. mutica* was placed in a Toby Teaboy with juveniles alone, the male was not observed harming them in any way. However, a male wanting to mate with the mother of these juveniles probably would not harm juveniles with the parental female absent if this system parallels that of lions (Maestripieri 1992). He may identify her absence and have no reason to harm juveniles. Perhaps a better way to test how conspecific males may pose a threat to juveniles is to tether the parental female to represent her presence to the male, but remove any protection she may be providing. Juveniles may stay close to the female, but if the male removes or harms them, the female will not be able to protect them. Since this is hypothesized for conspecific males and the threat from congeneric males is still

unknown, it is possible that females of *C. mutica* cannot distinguish between males of different species.

Aggression was highest in parental females, but was also present in non-brooding and ovigerous females. The only treatment that showed an absence of aggression was non-brooding females with other conspecific females, and aggression from ovigerous and parental females toward these individuals was minimal. Le Galliard et al. (2007) mentioned that for root voles (*Microtus oeconomus*), other females in a population may provide assistance in rearing young. If this were the case for *C. mutica*, females would not have a reason to attack conspecific females because they do not pose a threat to juveniles and fighting them would be disadvantageous.

Lastly, Montgomerie and Weatherhead (1988) discuss variation in maternal care intensity as a function of renesting potential: if a parent has the capability of producing more offspring in the reproductive season, parental care may be low, whereas if the parent will not have another chance to mate and produce offspring, parental care will be much higher. *C. mutica* has several broods throughout the summer and trials were run over the course of a few months, but a change in aggression with time was not observed in the data.

Percent of males fighting each other was highest in the presence of a non-brooding female, and male aggression was significantly higher with non-brooding females than the pooled data from male aggression with all other stimulus caprellids. This indicates that males recognize females in different reproductive states, and they recognize the presence of other males that may be competitors for a mate. However, it should be noted that the mean number of fights between males is not different when

parental females or non-brooding females were introduced. It may be difficult for males to distinguish between these two female reproductive states, since both of these treatments involved females without eggs or embryos in brood pouches. It is unknown how much time passes between juvenile emergence and the mother's next mating. Perhaps the receptiveness of parental females also contributed to the high variability in male aggression for this treatment. Additionally, recognition may only go as far as presence or absence of brooded eggs or embryos and not be associated always with male-male aggression.

I attempted to quantify receptivity by measuring developing oocytes in ovaries of non-brooding females used in this experiment. When non-brooding females' oocytes measure less than 150 μm, males do not attack each other. When females' oocytes measure 150-275 μm, fights between males are variable, ranging from 0-5 fights in 10 minutes. It is possible that at this oocyte size range, females are more likely to mate, and some males are able to identify and fight for those receptive females. The threshold size for developing oocytes ready for fertilization has not been reported in caprellids. There was one female whose oocyte size was larger than 275 μm, but males in this trial did not attack each other. This single case could represent a decline in aggression between males, which I cannot explain. It could also represent the same variation in male aggression in the presence of females with intermediate oocyte sizes.

Male-male aggression in response to mate access occurs in various other animals. In both Montagu's and Hen harriers, intraspecific male aggression peaks early in the breeding season when mates are chosen (Garcia and Arroyo 2002). Male-male interactions for mates seen in fig wasps often produce serious injuries and deaths,

reducing the number of competitors (Bean and Cook 2001). Lastly, in the amphipod, *Megalorchestia californiana*, male competition and female choice were investigated individually to determine the driving factor of males' reproductive success (Iyengar and Starks 2008). In this study, male-male competition was found to be a more important determinant of reproductive success than female choice. Larger males outcompeted smaller males for female access, while females chose equally among males varying in size (Iyengar and Starks 2008).

From the data presented for maternal care in *C. mutica*, I suggest that parental females recognize males of *C. drepanochir* and possibly males of *C. mutica* as threats to their offspring. Some aggression by females also occurs in their other reproductive states. For male-male aggression, I conclude that there is a recognition of non-brooding females and suggest that aggressiveness in males exists to obtain access to receptive females, but it is not consistent across all males of *C. mutica*.

BRIDGE

As presented above, aggression toward other caprellids is present in females of *Caprella mutica*. With all stimuli combined, there is a general trend of parental females exhibiting the highest number of attacks, non-brooding females being least aggressive, and ovigerous females having moderate aggression toward stimulus caprellids. When data are separated by stimulus caprellid, parental females show the highest number of fights toward males of both species, providing some evidence for a recognition system of males of both species as threats to juveniles. Male-male aggression in *C. mutica* is highest in

the presence of females in all three reproductive states, but attacks in the presence of non-brooding females were not significantly different from attacks in the presence of other females. Also, there is a trend of no aggression with small oocyte size and variable aggression with larger oocyte sizes. With these data, there is some support that the recognition system in male caprellids allows for identification of non-brooding females with larger oocyte sizes as receptive and males as competition for mates, although this recognition in males is largely variable.

The presence of male-male competition for mates allows for sexual selection to occur. Caprellids use their second gnathopods in fighting others, and the poison tooth, a sharp protrusion on the palmar face of the second gnathopods, could impose injuries to either of the aggressors (Caine 1991). Since evidence for this tooth being lethal in various species is conflicting, I continue in Chapter III to explore the poison tooth on *C. mutica* to determine if it is the cause of mortalities in male-male interactions.

CHAPTER III

USE OF A SPECIALIZED APPENDAGE IN THE CAPRELLID AMPHIPOD, *CAPRELLA MUTICA*: A CASE FOR WEAPONRY IN COMBAT?

INTRODUCTION

Male-male aggression and mate choice both control the outcome of sexual selection in many animals (Howard et al. 1997). In mate choice, females choose to mate with certain males due to their physical characteristics or behavior. With male-male aggression, males fight for females, and specific weaponry can be used to eliminate competition and ensure an individual's reproductive success (Kelly 2008). Low operational sex ratio (OSR: ratio of receptive females to sexually active males) can intensify aggression between males (Emlen and Oring 1977). Male parasitoid wasps, *Melittobia acasta*, demonstrated an increased number of fights and shorter lifespans with decreased OSRs, and male fig wasps, *Sycoscapter australis*, showed increased injuries at low OSRs (Bean and Cook 2001; Reece et al. 2007). Asynchronous sexual receptivity in females of a population can also increase the intensity of sexual selection (Emlen and Oring 1977). When females become receptive synchronously, mating time becomes the limiting factor, and an individual male is unable to mate with as many females as when females are receptive

asynchronously. With asynchronous female sexual receptivity, the OSR decreases considerably, and males are more likely to fight each other for mating opportunities.

Male gammarid amphipods have been shown to guard receptive females from the female's other potential mates (Birkhead and Pringle 1985; Borowsky and Borowsky 1987). Sexual selection studies performed on gammarid amphipods revealed that in *Hyalella azteca* and *Megalorchestia californiana*, larger males generally won male-male competitions and preferred larger, and thus, more fecund females (Wen 1993; Iyengar and Starks 2008).

Male-male aggression in competition for mates has also been reported in several caprellid amphipod species: *Caprella gorgonia* (Lewbel 1978), *C. laeviuscula* (Caine 1979), and *C. scaura typica* (Lim and Alexander 1986; Schulz and Alexander 2001). Although these behaviors were only observed in *C. gorgonia* and *C. laeviuscula*, Lim and Alexander (1986) experimentally showed decreased survival in males in the presence of receptive females. Caprellid amphipods use their second gnathopods in these aggressive interactions (Figure 1). Male *C. scaura typica* fight with the dactyl of their second gnathopod extended, which exposes the palmar surface of the propodus (Schulz and Alexander 2001). I have observed this posture in fights between males of *Caprella mutica* Schurin, 1935. There is a protrusion on the palmar surface of the second gnathopod in males, historically called the "poison tooth", and it is hypothesized by many to be used to injure or kill opponents during aggressive encounters (Caine 1979, 1991; Schulz and Alexander 2001). Poison teeth are present in several amphipod genera: *Caprella*, *Paracaprella*, *Luconacia*, *Paradicaprella*, and *Aciconula* (Schulz and Alexander 2001). While the poison tooth has been reported to cause injuries between

caprellids, there is no evidence that the tooth contains a toxin (Lewbel 1978; Caine 1991; Schulz and Alexander 2001). Scanning electron and light microscopy of the poison tooth in *C. scaura typica* showed groups of pores on the surface of the tooth in addition to numerous rosette glands within the second gnathopod with pathways to those pores (Schulz and Alexander 2001). I have observed pores like these on the surface of the second gnathopods of male *Caprella mutica* under a scanning electron microscope (Figure 2). Tests performed on *C. scaura typica* to identify secretions of the poison tooth were inconclusive (Schulz and Alexander 2001).

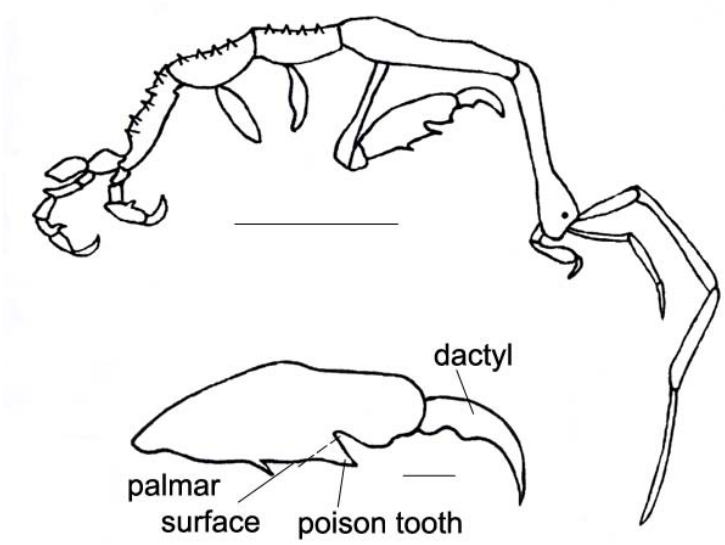

Figure 1: Line drawing of male *C. mutica*, missing pereopod 7, with an enlarged drawing of propodus and dactyl of the second gnathopod. Dashed line indicates where the poison tooth was detached. Scale bars indicate 5 mm for full caprellid and 1 mm for enlarged gnathopod.

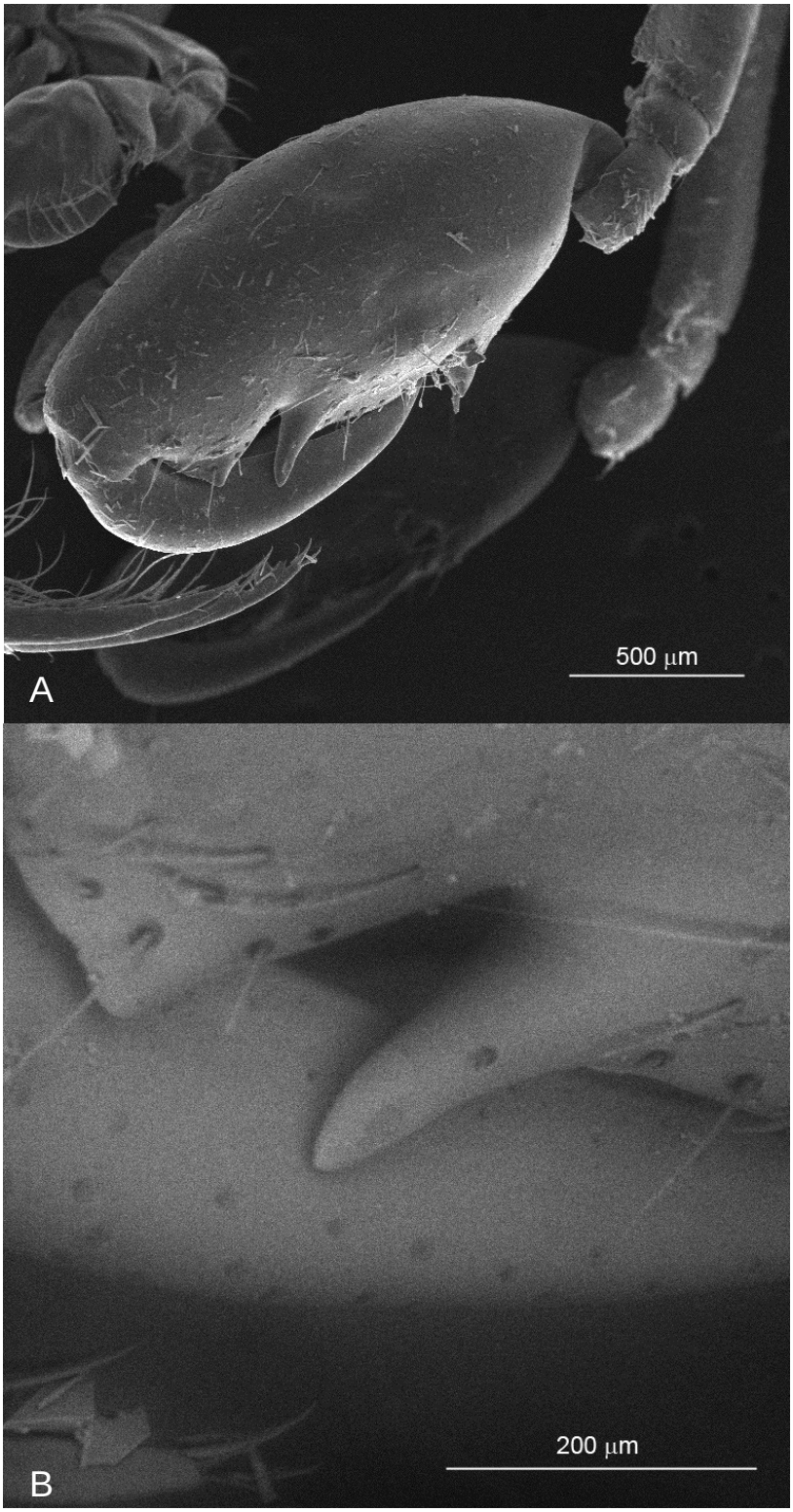

Figure 2: SEM micrographs of the A) gnathopod and B) poison tooth of a male *C. mutica*.

Since *C. mutica* possesses a poison tooth and exhibits similar behaviors to *C. scaura typica*, *C. mutica* may exhibit the same pattern of higher male mortality rates in the presence of receptive females. Although OSR has not been determined for populations of *C. mutica*, females (as a group) are continuously receptive throughout the summer, suggesting a possible influence of sexual selection. Males can grow much larger than females and often fight each other in the presence of a non-brooding female (Chapter II of this Thesis). In *C. mutica*, it is likely that increased male size or a better ability to injure competitors would lead to higher reproductive success. In this study, I test the hypothesis that the presence of the poison tooth increases the mortality rate in *C. mutica* male-male interactions by experimentally removing poison teeth from pairs of males competing for a non-brooding female.

METHODS AND MATERIALS

Caprella mutica were collected from red filamentous algae in the small boat basin, Charleston, OR. For each trial, two similarly sized males were placed in a Toby Teaboy (Aldridge Plastics, Aldridge, UK; 150-μm mesh netting) with a non-brooding female. Toby Teaboys were placed in a seatable with flowing seawater for 16-24 hours. In previous studies, receptive females have been defined as "about to molt" (Caine 1979; Lim and Alexander 1986). Since I was unable to distinguish particular stages of the molt cycle, non-brooding females were used to represent receptive females. All males used in the experiment were first anesthetized in 0.1% w/v MS222 for 10-15 minutes and measured (cephalon to abdomen) to ensure similar sizes within trials. The lengths of all

tested males ranged from 8.2-18.0 mm with an average of 12.0 ± 0.2 mm (SE) and the average difference in size for paired males was 0.09 ± 0.02 mm (SE). I removed poison teeth of anesthetized caprellids with a razorblade (see Figure 1). Males were allowed 2.5-4.25 hours after anesthetization to acclimate in Toby Teaboys within the seatable with flowing seawater. Females were then added to paired-male treatments. There were three paired-male treatments (n=11 in each treatment): 1 no-tooth male and 1 toothed male with a non-brooding female (N/T); 2 no-tooth males with a non-brooding female (N/N); and 2 toothed males with a non-brooding female (T/T). Two controls included 1 no-tooth male control (N) and 1 toothed male control (T). The physical condition of each caprellid was examined after 16-24 hours and mortality was recorded, along with which caprellid (toothed or no-tooth) had died. Mortality rates of toothed and no-tooth caprellids in each trial were compared using the goodness-of-fit G-test. Since G-tests are prone to Type I errors, the calculated statistic was adjusted by Williams correction factor (Williams 1976; Sokal and Rohlf 1987).

RESULTS

During attacks, males hit opponents with their second gnathopods. The dactyl is extended exposing the poison tooth on the palmar surface of the propodus. Mortality rates were highest (27.3%) in no-tooth males in the T/N treatment (Figure 3). Lower mortality rates were recorded for no-tooth males in N/N and N treatments (13.6% and 18.2%, respectively). Across all treatments, only one toothed male died, and it was in the treatment containing two toothed males. Mortality rates for toothed males in T/N and T

treatments were zero. Differences between all mortality rates were non-significant (G=0.76; p=0.98).

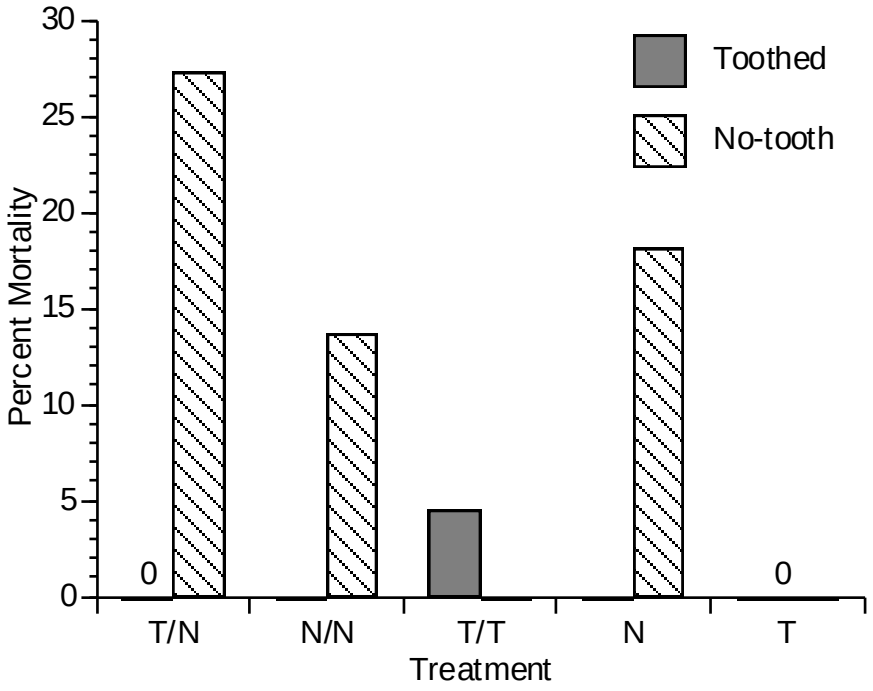

Figure 3: Mortality rates for paired males in the presence of a non-brooding female and males alone. T=toothed male; N=no-tooth (i.e. male with tooth removed). Percents are out of 11 trials for each treatment: 11 caprellids for N, T, and each of N/T; 22 for N/N and T/T. Values are not significantly different (G=0.76; p=0.98)

DISCUSSION

Due to the male's posture during fights, the poison tooth could be used to puncture, shove, or slice the opponent. Puncturing caprellids could injure or kill the opponent so that he is no longer able to compete. In either case, it is possible that a secretion is used to paralyze or kill the other male. I did not test for a toxin within the second gnathopods, but studies in other species are inconclusive (Lewbel 1978; Lim and Alexander 1986; Caine 1991; Schulz and Alexander 2001). Shoving could be used as a defensive behavior if the

caprellid is attacked by another. In slicing, one male could squeeze his opponent using the gap, located between the poison tooth and the main part of the propodus, and the dactyl of the second gnathopod to chop the opponent in half. Pores observed on the surface of the second gnathopod could be an exit point for a toxin and those with setae could be used as sensory structures in cuing the movement of the dactyl when something has entered the gap. The following conclusions from data collected were drawn as the tooth likely was used to puncture opponents to kill them.

Mortality was higher in no-tooth males than toothed males. Most of this can be attributed to the process of removing teeth from the second gnathopods, as mortality was high in controls containing a single no-tooth male. However, mortality in no-tooth males in N/T was higher than in N and was twice the mortality of no-tooth males in N/N. Although differences are non-significant, this trend suggests that poison teeth on one individual increases deaths of an opposing no-tooth male and that the poison teeth may be used for defending against an attacking male. Additionally, this can be supported by the mortality rates of toothed males. None of the toothed males in T/N and T treatments died. They were not expected to die in T, but these data suggest that no-tooth males cannot kill toothed males, either due to toothed males' defenses provided by the poison tooth or the inability of no-tooth males to kill other caprellids. Although the poison tooth may be used as a defense against attackers, there was some mortality in the T/T treatment, suggesting that the defense provided by the poison tooth is limited.

In this study, it seems mortality was mostly caused by the removal of teeth. The role of poison teeth on mortality may be minimal as their presence also may be used for defense. If the poison tooth also could be used as a shield, shoving opponents away, this

would provide evidence for defensive use of the tooth. Therefore, the use of the poison teeth as weapons on one caprellid and as defense on his combatant counteracts leading to low mortality. This was illustrated in the T/T treatment, where there was only one death.

For sexual selection via male-male aggression to take place, this aggression must be present in the species. For *Caprella mutica*, aggression is only seen in half of the males exposed to non-brooding females (Chapter II of this Thesis). If these data were applied to the current study, approximately 5 trials for each paired-male treatment would be affected by male-male aggression. If each of these 5 trials with males fighting resulted in the death of at least one caprellid, mortality rates would be much higher than reported in this study (expected 50% versus highest observed 27%). Therefore, it is possible that not all aggressive encounters end in the death of one of the combatants.

The data presented in this study support the conclusion that poison teeth on males play a part in either killing competitors for mates and/or defending themselves from other male caprellid attackers. However, it would be useful to perform further studies that have different methods of poison tooth removal. MS222 was more effective than magnesium chloride for anesthetization and has been suggested for work with other amphipods (Ahmad 1969). However, it may be advantageous to investigate other anesthetics as the use of MS222 combined with the removal of the tooth via razorblade led to much of the mortalities. In the case of tooth removal, I would recommend a more precise manner of detaching the poison tooth in micro-surgical removal. Observed mortality in this study may have been due to the combination of the MS222 and a large injury. In finding a method that reduces this mortality, clearer patterns may emerge.

Other additions to this study that may better investigate my question include close observations of male-male competitions and filling the gap between the poison tooth and gnathopod. With a high-speed camera, one may have the ability to see precisely how the poison tooth may be used in attacks between males. The use of the poison tooth to puncture, shove, or slice may be distinguished using this method. Additionally, glue may be used to fill the gap between the poison tooth and gnathopod, therefore removing the morphology of the tooth and gap without a surgical removal along with clogging any pores, which may emit a toxin. If this was used in an additional treatment, we could rule out the caprellid's ability to puncture, poison, or slice his opponent.

In males of *Caprella mutica*, there is some aggression in the presence of a non-brooding female, and they use their second gnathopods in aggressive encounters. Although mortalities were not significantly different, patterns in data from this study support the conclusion that the poison tooth located on the second gnathopods could be used to cause fatalities in these attacks or as a defense from their attackers. The presence of a toxin within the tooth was not investigated, but if present, could aid a male in his reproductive success by reducing his competition for mates.

CHAPTER IV

CONCLUDING SUMMARY

The purpose of this thesis was to quantify aggressive behaviors and explore the use of a specialized appendage as a form of sexual selection in *Caprella mutica*. Aggressive maternal care behaviors, largely studied in vertebrates and insects, have been investigated in a handful of caprellids species that do not include *C. mutica*. Male aggressive behaviors have various functions depending on the animals' life history and have been observed, but generally not quantified, in caprellid species. The presence of aggression between males leads to questions about the method of injury or death. Previous studies have cited the "poison tooth," a protrusion on the second gnathopod, as the cause of deaths during conflicts between male caprellids.

In Chapter II, I described observed caprellid behaviors and quantified female aggression against other caprellids. I compared aggression of females in three different reproductive states with four different stimulus caprellids: conspecific and congeneric males and females. When treatments were grouped by the reproductive state of the tested female, both the number of fights per female and the percent females fighting showed the same pattern: parental females exhibited the most aggression, followed by the ovigerous females, then non-brooding females. When the total number of fights was categorized by type of stimulus caprellid, parental females were more aggressive toward males of both

species than females of both species. From this, I conclude that parental females may identify conspecific and congeneric males as potential threats to their juveniles. If these kill juveniles, conspecific males may make the parental females receptive to mating earlier than if juveniles dispersed naturally. It is possible that congeneric males kill juveniles because they are competing for food or that the males eat the juveniles; however, there is no evidence for either of these explanations.

In the next part of Chapter II, I investigated male-male aggression between two male caprellids presented with another male or a female caprellid in one of three reproductive states. Males showed high aggression in the presence of females in all three reproductive states, but aggression was still present in treatments with males only. The percent of males fighting was highest in the presence of a non-brooding female, which suggests that they may recognize non-brooding females as receptive and therefore identify males as competitors for mates. When non-brooding females had small oocytes within their ovaries, males showed no aggression. However, when females' oocyte sizes were larger, male-male aggression was present, but variable. Due to the absence of aggression between males in some trials, it is possible that not all males recognize females with large oocytes as receptive.

In Chapter III, I examined the potential use of weaponry in males of *C. mutica*. Given that males fight each other with their second gnathopods in the presence of non-brooding females and the poison tooth has been cited as the possible cause of death in aggressive encounters, I tested if the presence of the poison teeth caused greater mortality rates in their combatants. The result of this experiment showed higher mortality rates of males with poison teeth removed, even in treatments containing one male. Therefore, the

method of removing poison teeth was the likely explanation for much of the high mortality. However, no-tooth males showed twice the mortality when paired with a toothed male (N/T) than when paired with another no-tooth male (N/N). The differences of no-tooth male mortalities between these treatments suggest that toothed males did have an effect on the mortalities of no-tooth males. The methods used to remove poison teeth clearly played some part in the mortalities recorded, but future studies with improved methods could be used to further investigate sexual selection in *C. mutica*. The combination of aggressive behaviors and the use of weaponry likely influence morphological features in *C. mutica*.

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